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1. The project to evaluate "Thermal Sterilization Processes for Unmanned Landers" was initiated at the request of the Planetary Quarantine Officer. The system consists of a temperature-controlled oven with a nitrogen gas supply containing a known concentration of water. Water vapor during the sterilization cycle may be controlled. Moisture analyzers with an accuracy of ±5% of full scale are utilized to monitor the gas flowing over spore samples contained in the oven. A temperature controller was developed and installed which gives flexibility to temperature-time sterilization cycles. For purposes of calibration, the oven was filled with 2100 empty cups and the temperature raised to 150 C for a minimum of 6 hours then allowed to cool to room temperature. The sample cups were then removed, nutrient media were added to each cup, and allowed to incubate for 7 days. The cups were then inspected and scored for growth. Three replicate experiments were conducted with no positives observed. These experiments fulfilled the requirement for aseptic technique. Two experiments were performed to test the homogeneity of the model terminal sterilization cycle oven by loading the oven in the conventional manner with 70 trays of 29 cups each. The top shelf and bottom shelf were loaded with 24 trays each and the middle shelf with 22 trays. Each cup was inoculated with approximately 10^5 spores of B. subtilis var. niger, and placement of trays was made on the basis of a random number chart. The oven was closed and purged with dry nitrogen (mil. spec.) overnight. The oven was then manually programmed from room temperature to 120 C over an approximate 7 hour period, held for 1 hour, and allowed to cool for 2 hours. The integrated lethality of the program was equal to 3.5 hours at 120 C. The results showed that the oven was homogeneous.

   Sixteen aluminum trays (7" wide x 74" long x 40" high) containing two 3" x 72" ribbons of teflon (5 mil FEP Dupont) were located in the Low Bay area of the Manned Spacecraft Operations Building (MSOB), a class 100,000 clean room. The teflon ribbons were cleaned in the same manner as stainless steel strips described in "NASA Standard Methods for the Microbiological Examination of Space Hardware," NHB 5340.1A October 1968. New ribbons were preconditioned by two exposures to dry heat for 3 hours at 175 C prior to a third heating interval for sterilization. Ribbons were sterilized before exposure in 250 ml beakers covered with aluminum foil. Ribbons were exposed to the clean room environment for 7 days, following which each ribbon was rolled up and aseptically inserted into a sterile stainless steel holder, and placed into sterile 600 ml beakers covered with aluminum foil. When fiberglas cloth (3" x 72") was used as a "spacer," a sterile fiberglas cloth was placed over the exposed teflon ribbon, and a teflon ribbon with fiberglas cloth was rolled and treated as described above. Aluminum trays were wiped with membrane filtered 70% isopropyl alcohol after exposed ribbons were collected. While handling the teflon ribbons, personnel used clean room garments and sterile gloves.

   Control and test ribbons were selected randomly and 8 of the 32 ribbons were used for N_0 determinations. The relative humidity and temperature of the assembly area were monitored constantly. The recovery medium was TSA broth (same formula as TSA except without agar) plus 0.2% yeast extract and 0.1% soluble starch; TSA plus yeast extract and starch was used for determining total and spore counts on control ribbons. Each control ribbon was rolled up and placed in a sterile jar, 400 ml of sterile rinse solution (.002% Tween 80) was added and the jars insonated for 6 minutes, removed, manually shaken 25 times and then insonated again for 6 minutes. Five ml portions were plated in duplicate for total counts, and duplicate 50 ml portions were heat shocked

(80 C, 25 min) and plated for spore counts. Plates were incubated at 32 C and colonies counted after 2, 3, and 7 days. Test ribbons were placed in the thermal oven from front to rear in the order of retrieval from the clean room. Each oven shelf contained eight test ribbons plus three sterile control ribbons. A total of 24 test ribbons and 9 sterile control ribbons were used per run. Test ribbons and sterile control ribbons were placed in the oven and exposed to dry nitrogen, as specified in paragraph 2.2.2 of NHB 8020.12, 6 to 8 hours prior to start of heat cycle. Sterile forceps were used in handling the ribbons into and out of the oven. After the thermal treatment, each ribbon with stainless steel holder was placed into a sterile jar and 400 ml of sterile broth was added aseptically. The jars were incubated at 32 C and observed for the presence of growth at 7 days and weekly up to 28 days. The sterile control ribbons were treated in the same manner as the test ribbons. During assay of ribbons, personnel wore clean room gown, hat, sterile gloves and mask. All survivors were isolated in pure cultures and identified.

The thermal oven was located in a vertical laminar flow clean bench which was attached to a horizontal laminar flow clean bench. The two benches were enclosed by a plexiglas canopy and plastic curtains. In essence, the thermal experiments and assays were done in a small clean room under class 100 hoods.

The thermal experiments were conducted in a dry heat oven under the following conditions:

1. Volume of oven - 1.5 cubic feet.
2. Nitrogen flow rate to oven during thermal experimental runs - 6 CF/hr or approximately 3 L/min. Between runs, the oven is purged with dry nitrogen.
3. Moisture content of dry nitrogen used - approximately 0.004 mg/L (5 ppm) (MIL-P-27401B).
4. Moisture content of nitrogen during thermal runs - 1.2 mg/L (1500 ppm).
5. Thermal Profile used - High Thermal Inertia (Figure 1).

All thermal experiments were conducted using the proposed sterilization cycle for unmanned landers (Figure 1). Preliminary experiments were conducted using Bacillus subtilis var. niger spores as the test microorganism to evaluate the sterilization cycle. Spores were inoculated directly onto sterile teflon ribbons, or on sterile fiberglas used as a "spacer." Stainless steel cups also were inoculated and exposed to the thermal cycle. The results are shown in Table 1. The thermal resistance of naturally-occurring airborne bacterial spores collected on exposed teflon ribbons in the MSOB clean room are shown in Table 2. With the exception of two experiments, survivors were recovered from all thermal experiments. The use of teflon ribbons only, or teflon with fiberglas did not affect the number of survivors. Extending the heating time from 5 to 15 additional hours at temperature failed to achieve sterilization. Pretreatment of teflon ribbons at 85 C for two hours, prior to subjecting them to the heat cycle, also was unsuccessful.

Table 3 shows the results when the overall water content in the oven was reduced from approximately 1500 ppm (v/v) to 6-12 ppm at the 113 C high thermal inertia cycle. Although survivors were recovered in one experiment, not enough data has been obtained to assess the effect of this change on the effectiveness of the cycle.

Heat survivors were isolated and identified. In all experiments, the survivor from each positive ribbon was found to be a pure culture. A listing of the identification of the heat survivors are shown as follows:

Microorganism	No. of Times Recovered
Bacillus lentus	22
Bacillus brevis	4
Bacillus coagulans	1
Atypical Bacillus spp.	15
Actinomycete	1

Of the 42 bacillus isolates which survived the thermal exposure, 41 were found to be unable to grow anaerobically, the exception being the isolate identified as B. coagulans. Generally, heat survivors were found to have the following characteristics:

1. Produce smaller colonies and grow much slower on laboratory media than non-heat stressed environmental isolates of the Genus Bacillus.
2. Sporulate poorly on sporulation media.
3. Biochemically less active than non-heat stressed environmental isolates.

Studies are presently being conducted to determine if the above characteristics are inherent properties of the organisms or are the result of thermal exposure.

At the request of the Planetary Quarantine Advisory Panel, N_0 values for all of the teflon ribbon experiments to date (569 ribbons) were analyzed with the following results:

1. Mean - 248.1
2. Median = 190.0
3. Standard Deviation = 286.4
4. 95% Confidence Limits = 225-272
5. 99% Confidence Limits = 217-279
6. Coefficient of Variation = 1.15

2. Identification of the microorganisms isolated from the Pioneer G spacecraft was completed (Table 4). Greater than 50% of the microorganisms were of the types associated with soil and dust.

TABLE 1. THERMAL RESISTANCE OF B.G. SPORES EXPOSED TO THERMAL TREATMENT (ALL AT RELATIVE HUMIDITY OF 0.133%)*

Experiment Number	Temperature Cycle	N_0 Spores	Incubation Time (Days)	Positive/Total	MPN For Ribbon	Controls Positive/Total
1[a]	A	3.6×10^5	28	3/24	0.134	0/8 (Controls not placed in oven)
2	A	1.8×10^5/cup	14	0/1972	0.0	0/60 cups (Controls not placed in oven)
3[b]	A	1.9×10^5	28	0/32	0.0	0/8 (Controls not placed in oven)
4[b]	A	2.6×10^5	28	0/32	0.0	0/8 (Controls not placed in oven)
5[c]	A	1.4×10^5	28	0/24	0.0	0/8 (Controls not placed in oven)
6[c]	A	3.3×10^5	28	0/33	0.0	0/0
7[c]	A	1.8×10^5	28	0/33	0.0	0/0
8[c]	A	1.2×10^5	28	0/33	0.0	0/0

No specified controls used

A - Thermal Profile 113 C - High Thermal Inertia (See Figure 1)
[a] B.G. spores were inoculated on Fiberglas
[b] B.G. spores were inoculated on Teflon Ribbon. Fiberglas used as "Spacer."
[c] B.G. spores were inoculated on Teflon Ribbon. Fiberglas used as "Spacer" for 12 ribbons.
* Relative Humidity - 0.133% is equivalent to 1.2 mg/L or 1500 ppm of water

TABLE 2. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY (ALL AT RELATIVE HUMIDITY OF 0.133%)*

Experiment Number	Temperature Cycle	N_0 Spores	Incubation Time (Days)	Positive/Total	MPN For Ribbon	Controls Positive/Total
2	A	1.4×10^2	28	1/24	0.042	0/9
5	A	4.2×10^2	28	1/22	0.047	0/9
6	A	4.2×10^2	28	6/24	0.287	0/9
7	A	5.3×10^2	28	1/12 (Teflon only)	0.087	0/9
			28	1/12 (Teflon/fiberglas)	0.087	
8	A	5.8×10^2	28	1/12 (Teflon only)	0.087	0/9
			28	2/12 (Teflon/fiberglas)	0.182	
9	A	3.5×10^2	28	3/12 (Teflon only)	0.287	0/9
			28	1/12 (Teflon/fiberglas)	0.087	
10	A	2.2×10^2	28	6/24	0.287	0/9
11	A	1.8×10^2	28	0/24	0.0	0/9
12	A	2.0×10^2	28	0/24	0.0	0/9
13	B	1.6×10^2	28	1/24	0.042	0/9
14	A	1.4×10^2	28	1/24	0.042	0/9
15	C	7.9×10^2	28	1/24	0.042	0/9
16	A	2.4×10^2	28	3/24	0.134	0/9
17	D	1.5×10^2	28	1/24	0.042	0/9
18	E	2.1×10^2	28	4/24	0.182	0/9

A - Thermal Profile 113 C - High Thermal Inertia (See Figure 1)
B - Thermal Profile 113 C - Extended 5 hours at temperature (Total time at temperature 35 hours)
C - Thermal Profile 113 C - Extended 10 hours at temperature (Total time at temperature 40 hours)
D - Thermal Profile 113 C - Extended 15 hours at temperature (Total time at temperature 45 hours)
E - Thermal Profile 113 C - Teflon ribbons pretreated at 85 C for 2 hours prior to start of High Thermal Inertia cycle
* Relative Humidity - 0.133% is equivalent to 1.2 mg/L or 1500 ppm of water

TABLE 3. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY (ALL AT RELATIVE HUMIDITY OF 0.001%)*

Experiment Number	Temperature Cycle	N_0 Spores	Incubation Time (Days)	Positive/Total	MPN For Ribbon	Controls Positive/Total
19	A	3.7×10^2	28	0/24	0.0	0/9
20	A	2.1×10^2	28	2/24	0.087	0/9
21	A	2.8×10^2	28	0/23	0.0	0/9
22	A	2.3×10^2	28	0/24	0.0	0/9
23	A	1.0×10^2	21	0/24	0.0	0/9
24	A	1.3×10^2	14	0/24	0.0	0/9

A - Thermal Profile 113 C - High Thermal Inertia (See Figure 1)

* Relative Humidity - 0.001% is equivalent to 0.01 mg/L or approximately 5-12 ppm of water

TABLE 4. TYPES AND NUMBERS OF MICROORGANISMS DETECTED FROM PIONEER G SPACECRAFT ON TRYPTICASE SOY AGAR

	Microorganisms Isolated		
	Aerobes[1]	Anaerobes[2]	Aerobic Spores[3]
Staphylococcus spp.			
Subgroup III	1	2	0
Subgroup IV	4	6	0
Micrococcus spp.			
Subgroup 1	3	3	0
Subgroup 7	3	0	0
Bacillus spp.			
B. alvei	1	0	0
B. brevis	1	0	0
B. cereus	1	0	0
B. circulans	0	1	0
B. lentus	3	0	2
B. macerans	1	0	1
B. megaterium	0	0	1
B. pumilus	1	0	0
Yeasts	2	0	0
Molds	3	0	0
Atypical *Bacillus* spp.	6	1	1
NUMBER ISOLATED	30	13	5

[1] Microorganisms isolated from culture plates incubated aerobically

[2] Microorganisms isolated from cutlure plates incubated anaerobically

[3] Microorganisms isolated from heat-shocked samples, incubated aerobically

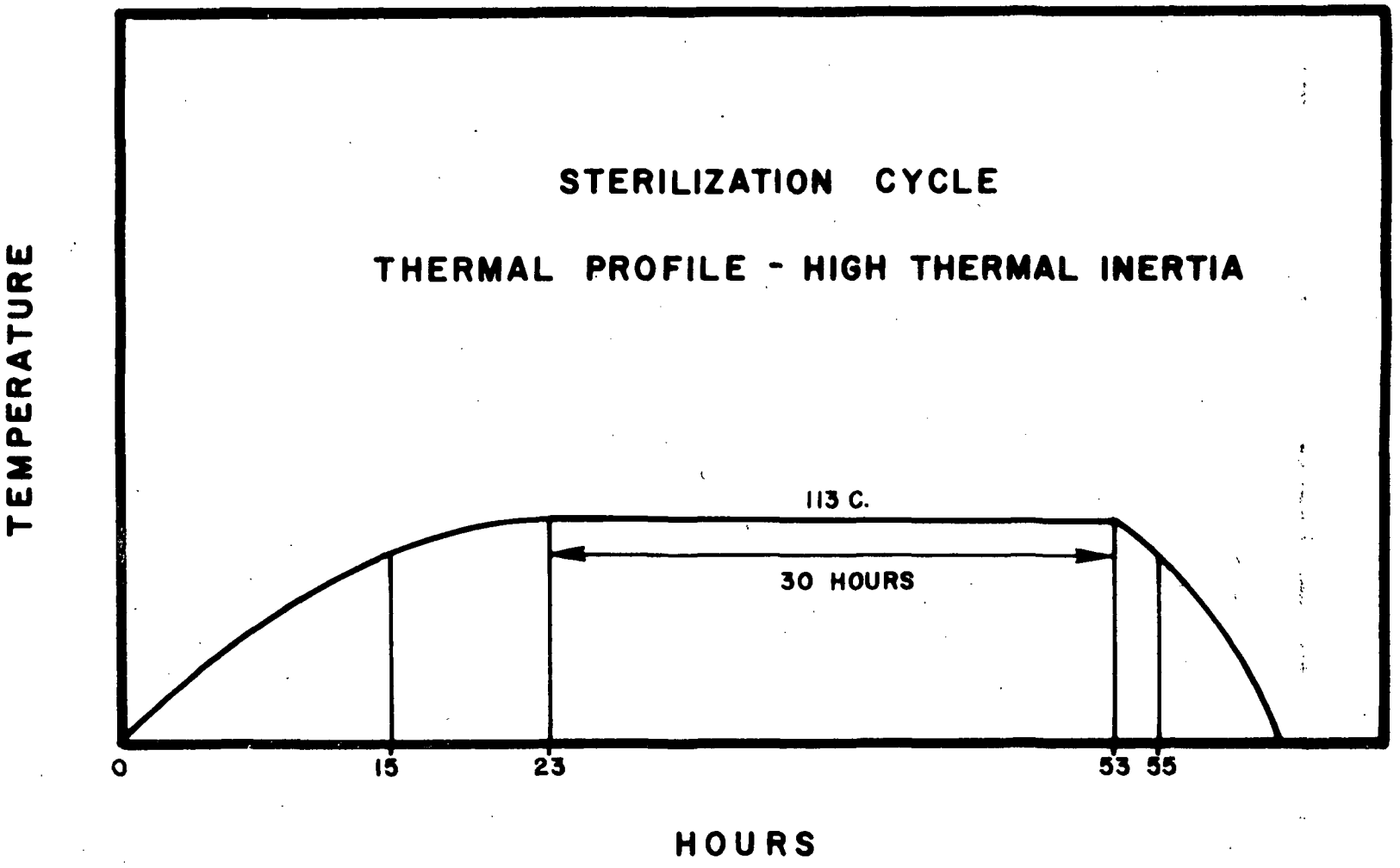

FIGURE 1. PROPOSED STERILIZATION CYCLE FOR UNMANNED LANDERS